

Interim Progress Report

**ECOLOGICAL RISK ASSESSMENT OF PERCHLORATE ON FISH, AMPHIBIANS, AND MAMMALS
IN THE LAKE BELTON AND LAKE WACO WATERSHEDS**

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I. Introduction

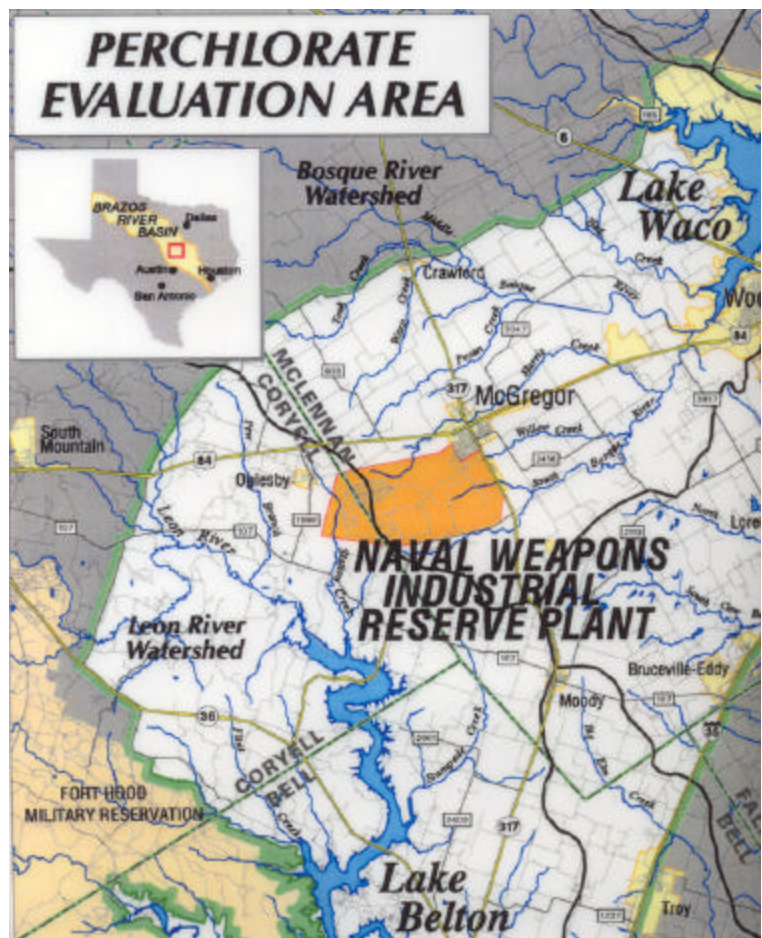
The technical objective of this research is to assess environmental impacts of perchlorate contamination in riparian areas within the Lake Waco and Lake Belton watersheds. Indigenous plant, fish, amphibian, avian, and mammalian species are being examined as potential receptors and vectors of perchlorate movement through food webs by characterizing perchlorate bioavailability across trophic levels and evaluating the toxicological impacts of perchlorate on exposed biota. Perchlorate exposure is also being linked with appropriate toxicological endpoints developed in laboratory studies including thyroid histology and hormone concentrations. Data generated from these studies are being coupled to models of contaminant movement and physiological-based pharmacokinetic (PBPK) models to better describe the ecological risks to individual organisms associated with perchlorate contamination.

II. Points and Pathways of Exposure

Water

Historical data and several months of periodic sampling of surface water in an around the study area has provided some details as to where perchlorate exposures have the highest potential to occur:

Station Creek south of NWIRP, the spring on Oglesby Road, Harris Creek from Highway 84 to areas east of McGregor, and 2 un-identified tributaries to the South Bosque near Highway 317. The highest perchlorate concentrations (range = 67 - 540 ppb) are consistently observed in one of the un-identified tributaries to the South Bosque near Highway 317. Overall, perchlorate **contamination of surface water within the study area varies with location, season, and rainfall conditions**, with the exception of the spring on Oglesby Road (range = 26 - 67 ppb). Most of these surface water bodies are surrounded by vegetation (likely exposure pathway to terrestrial animals) and support aquatic life (potential exposure pathway to humans). The spring on Oglesby Road has surface water flow year-round, potentially serving as a source of exposure even during drought periods. Property access has



indicated that larger mammals (cattle) also use the spring water on this site, as well as areas surrounding Station Creek near the NWIRP property.

Sediment

Sediment sampling at the exposure points identified above indicates that perchlorate is also present in sediments. These sediments serve as a potential source of perchlorate to overlying water because perchlorate concentrations in sediment do not fluctuate as greatly as some surface water concentrations. **Perchlorate is persistent in sediment** and its persistence varies with available nitrate as microbial degradation proceeds with perchlorate being utilized as an electron acceptor only after available nitrate is used. Sediments also serve as a potential exposure point for perchlorate to (1) submergent and emergent vegetation and (2) lower trophic level organisms (earthworms, insects), both of which serve as food items for larger fish, birds, and some small mammals.

Vegetation

Both laboratory and field studies indicate that perchlorate is readily taken up by aquatic and terrestrial vegetation. In addition, **perchlorate has been found in various plant species** near the exposure points identified above. These plants have included algae, riparian vegetation (e.g. smartweed) in or near the water, and trees. Monitoring data indicate that terrestrial plants have significant (>1 ppm on a dry weight basis) concentrations of perchlorate, even plants more than 9 m from stream water containing perchlorate. It is possible that these trees are taking up perchlorate from groundwater and not from surface water. The pathway of perchlorate exposure to higher terrestrial and aquatic organisms passes through vegetation and algae.

A market basket-type residue survey focusing on typical garden vegetables is planned for the upcoming year. This survey will address a current data gap for potential human exposure related to the use of perchlorate-contaminated water for irrigation. Additional data gaps related to plants that will be addressed in the coming year include (1) re-release/leaching potential of perchlorate from plant tissues and leaf litter, and (2) transformation rates of perchlorate in vegetation. These data will provide a more accurate assessment of perchlorate sources, spatial distribution, and risk.

Fish

Both laboratory and field data indicate that **human exposure to perchlorate through consumption of fish caught in the study watersheds is highly unlikely**. This conclusion is based on (1) where perchlorate occurs in surface water and the size of fish supported by those streams, (2) the preferential accumulation of perchlorate in rarely consumed tissue (head) rather than the fillet, (3) fillet residue data on larger fish caught near the exposure points and the lakes, and (4) laboratory studies on perchlorate tissue distributions in exposed fish. Additional sampling of fish during the

coming year will supplement these data and address a data gap related to the temporal robustness of the current data set.

Terrestrial Animals

Identification of exposure pathways to and through terrestrial animals (small mammals, birds) has been hampered by limited property access and a lack of suitable habitat in areas identified as being susceptible to perchlorate contamination. Initial efforts at avian sample collection focused on tissues likely to be consumed by humans. A limited number of breast tissue samples from doves (mourning and white-wing) collected last fall (2001) near Harris Creek at Highway 84 were negative for perchlorate. However, more recent residue data indicate that certain areas within the study boundary (the spring on Oglesby Road, Station Creek, and the 2 Un-identified tributaries to the South Bosque) pose **a significant exposure risk to resident and migratory terrestrial animals**, although these animals are not likely to be consumed by humans. Perchlorate has been detected in tissues (kidney, liver) of various small mammals (deer mice, white-footed mice, harvest mice, house mice, and cotton rats) and small birds at concentrations >2 ppm (μg perchlorate / g tissue dry weight).

In general, higher concentrations of perchlorate were detected in kidneys than in livers, perhaps indicating a recent or intermittent exposure since kidneys remove, store, and eliminate ions from blood to maintain osmotic balance. However, the relationship between kidney and liver levels of perchlorate lacked consistency. This has been identified as a data gap, and laboratory studies on perchlorate distribution in tissues of small mammals are currently underway. An additional data gap identified is perchlorate exposure to larger mammals (especially cattle). Cattle grazing is extensive in the study area, including around the exposure points described previously, and these cattle could also serve as a pathway for perchlorate exposure to humans.

III. Impacts on Ecological Health

Amphibians

The rationale for examining amphibians is that, as a group, these animals are exquisitely sensitive to perchlorate and subsequent disruption of thyroid function. If there were significant risk to human health and wildlife caused by perchlorate contamination of surface waters, amphibians, particularly developing amphibians, would be the first group to indicate problems with thyroid function. We collected adult and larval frogs from reference and contaminated sites in the study area and preserved those specimens for subsequent histological assessment of thyroid function. Thyroid histopathology revealed **no noticeable abnormalities** that could be related to perchlorate, including observations in animals collected from known perchlorate-contaminated sites. Healthy populations of several frog species exist, and developing frogs that have been collected show signs of normal development. Again, this includes frogs living adjacent to perchlorate-contaminated sites.

Second, we collected water samples from reference and contaminated sites, and transported those samples back to the laboratory for use in EDSTAC bioassays. Larval frogs were raised in these water samples to determine if thyroid-disrupting agents, including perchlorate, were present. All frogs raised in these water samples have exhibited **normal metamorphosis**, except for frogs in one water sample from the South Bosque in which the animals repeatedly died over the course of three trials. These water samples are being tested further. The lethality of the water is probably not associated with perchlorate, as laboratory studies indicate that perchlorate is lethal only at very high (> 1000 ppm) concentrations.

Given the transient nature of perchlorate concentrations at most of the exposure points identified, we will continue with thyroid histopathological assessment of frogs collected from surface waters in the study area during the upcoming year. These assessments will ensure that the results obtained thus far are not an anomaly. The Tier I EDSTAC tests performed thus far are useful for detecting impacts at high concentrations, but may not be sensitive enough to detect the impacts of low perchlorate concentrations. Therefore, we will also perform full developmental period metamorphosis tests using water collected from the study area.

Fish

Histological analysis is also being used to evaluate the impact of perchlorate on fish in the study area. The indices of thyroid dysfunction include thyroid follicle cell hyperplasia, hypertrophy (increased epithelial cell height), and depleted colloid within the follicle. In fish, the thyroid tissue is present as scattered follicles. Follicles are balls of epithelial cells with a mass of thyroid hormone – the colloid – in the center. When thyroid hormone synthesis is disrupted, the colloid becomes depleted, the epithelial cells grow in height, and some cells undergo hyperplasia (increased cell division). Data collected in year one indicated that the **thyroid tissues of fish from Harris Creek and Station Creek are being affected**, most likely by perchlorate. Fish from Coryell Creek show the least effects. Thyroid follicles from fish at Wasp Creek seem to be slightly affected, although there has been no perchlorate detected there. Percent hyperplastic follicles and follicles with depleted colloid seem to be a better indicator of perchlorate impacts in fish than follicle cell height. Continued evaluation of these indices in the context of known effects observed in laboratory studies will be a focus during the upcoming year.

Terrestrial Mammals

Additional sampling of terrestrial species will be necessary to more accurately delineate the spatial extent of exposure among these organisms, and to assess potential adverse effects. Currently, **there are no data available to evaluate potential effects in mammals** related to the tissue concentrations observed to date. Future field research will focus on effects of perchlorate on exposed birds and rodents, as well as additional species of potential human health or ecological significance.

including raccoons, squirrels, rabbits, and cattle. Recently, property owned by the Texas A&M University System has become accessible, which will produce additional study areas near the NWIRP boundary. Moreover, laboratory studies (tissue distributions of perchlorate, etc.) designed to compliment or verify field data are currently underway.

IV. Data Integration and Assessment of Risk

Modeling efforts during the first year have yielded several models relevant to evaluating the potential impacts of perchlorate on wildlife including (1) a model of small mammal uptake of perchlorate, (2) a model of terrestrial plant uptake, (3) a model of fish uptake of perchlorate, and (4) a model of algae growth. Current model development efforts are also addressing uptake of perchlorate in avian species, and aquatic plant uptake.

Modeling results for the small mammal model illustrate our modeling approach. The model has been programmed in Matlab® and Fortran and is being be run on the Cray Origin 2400. The model consists of two submodels: (1) a physiologically based pharmacokinetics (PBPK) model of the uptake and distribution of perchlorate in small mammal body tissues for each individual in the population, and (2) a model of the thyroid hormone secretion and distribution as affected by the perchlorate concentration at the thyroid. The model is stochastic in that it contains random variables for the concentrations of perchlorate in drinking water and residues in other diet components. These random variables provide the capability to conduct Monte Carlo simulations for various perchlorate exposure scenarios. The model is used to predict the dynamics of perchlorate uptake and distribution, based on stochastic feeding rates and elimination rates, and the effect of perchlorate on thyroid function. Several individuals can be simulated to develop probability distributions. Modeling efforts during the upcoming year will focus on completing current models, calibrating the models using field and laboratory data, and integrating a model of fish consumption in humans.